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UNITED STATES PATENT APPLICATION

OF

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FOR

MAMMALIAN ZCYTOR11


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SUMMARY OF THE INVENTION

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binding receptor polypeptide. The polypeptide comprises a sequence of amino acids selected from the group consisting of (a) residues 18 to 228 of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 80% identical to (a) or (b). Within one embodiment, the polypeptide comprises residues 18 to 228 of SEQ ID NO:2. Within another embodiment, the polypeptide encoded by the isolated polynucleotide further comprises a transmembrane domain. The transmembrane domain may comprise residues 229 to 251 of SEQ ID NO:2, or an allelic variant thereof. Within another embodiment, the polypeptide encoded by the isolated polynucleotide further comprises an intracellular domain, such as an intracellular domain comprising residues 252 to 574 of SEQ ID NO:2, or an allelic variant thereof. Within further embodiments, the polynucleotide encodes a polypeptide that comprises residues 1 to 574, 1 to 251, 1 to 228, 18 to 251 or 18 to 574 of SEQ ID NO:2. Within an additional embodiment, the polypeptide further comprises an affinity tag. Within a further embodiment, the polynucleotide is DNA.

Within a second aspect of the invention there is provided an expression vector comprising (a) a transcription promoter; (b) a DNA segment encoding a ligand-binding receptor polypeptide, wherein the ligand-binding receptor polypeptide comprises a sequence of amino acids selected from the group consisting of: (i) residues 18-228 or any one of the residues described above of SEQ ID NO:2; (ii) allelic variants of (i); and (iii) sequences that are at least 80% identical to (i) or (ii); and (c) a transcription terminator, wherein the promoter, DNA segment, and terminator are operably linked. The ligand-binding receptor polypeptide may further comprise a secretory peptide, a transmembrane domain, a transmembrane domain and an intracellular domain, or a secretory peptide, a transmembrane domain and an intracellular domain.

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Within a third aspect of the invention there is provided a cultured eukaryotic cell into which has been introduced an expression vector as disclosed above,
5 wherein said cell expresses a receptor polypeptide encoded by the DNA segment. Within one embodiment, the cell further expresses a necessary receptor subunit which forms a functional receptor complex. Within another embodiment, the cell is dependent upon an exogenously supplied
10 hematopoietic growth factor for proliferation.

Within a fourth aspect of the invention there is provided an isolated polypeptide comprising a segment selected from the group consisting of (a) residues 18 to
15 228 of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 80% identical to (a) or (b), wherein said polypeptide is substantially free of transmembrane and intracellular domains ordinarily associated with hematopoietic receptors. Additional
20 polypeptides of the present invention include Within one embodiment, the polypeptide comprises residues 18 to 228 of SEQ ID NO:2. Within another embodiment, the polypeptide further comprises a transmembrane domain. The transmembrane domain may comprise residues 229 to 251 of
25 SEQ ID NO:2, or an allelic variant thereof. Within another embodiment, the polypeptide further comprises an intracellular domain, such as an intracellular domain comprising residues 252 to 574 of SEQ ID NO:2, or an allelic variant thereof. Within further embodiments the
30 polypeptide that comprises residues 1 to 574, 1 to 251, 1 to 228, 18 to 251 or 18 to 574 of SEQ ID NO:2.

Within one embodiment, the polypeptide further comprises an immunoglobulin F_C polypeptide. Within a
35 another embodiment, the polypeptide further comprises an affinity tag, such as polyhistidine, protein A,

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glutathione S transferase, or an immunoglobulin heavy chain constant region.

Within a further aspect of the invention there
5 is provided a chimeric polypeptide consisting essentially
of a first portion and a second portion joined by a
peptide bond. The first portion of the chimeric
polypeptide consists essentially of a ligand binding
domain of a receptor polypeptide selected from the group
10 consisting of (a) a receptor polypeptide as shown in SEQ
ID NO:2; (b) allelic variants of SEQ ID NO:2; and (c)
receptor polypeptides that are at least 80% identical to
(a) or (b). The second portion of the chimeric
polypeptide consists essentially of an affinity tag.
15 Within one embodiment the affinity tag is an
immunoglobulin F_C polypeptide. The invention also
provides expression vectors encoding the chimeric
polypeptides and host cells transfected to produce the
chimeric polypeptides.

20
The present invention also provides for an
isolated polynucleotide encoding a polypeptide selected
from a group defined SEQ ID NO:2 consisting of residues 1
to 228, residues 1 to 251, residues 1 to 574, residues 2
25 to 228, residues 2 to 251 and residues 2 to 574. Also
claimed are the isolated polypeptide expressed by these
polynucleotides.

The invention also provides a method for
30 detecting a ligand within a test sample, comprising
contacting a test sample with a polypeptide as disclosed
above, and detecting binding of the polypeptide to ligand
in the sample. Within one embodiment the polypeptide
further comprises transmembrane and intracellular domains.
35 The polypeptide can be membrane bound within a cultured
cell, wherein the detecting step comprises measuring a
biological response in the cultured cell. Within another

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embodiment, the polypeptide is immobilized on a solid support.

Within an additional aspect of the invention
5 there is provided an antibody that specifically binds to a polypeptide as disclosed above, as well as an anti-idiotypic antibody which binds to the antigen-binding region of an antibody to Zcytor11.

10 In still another aspect of the present invention, polynucleotide primers and probes are provided which can detect mutations in the Zcytor11 gene. The polynucleotide probe should at least be 20-25 bases in length, preferably at least 50 bases in length and most
15 preferably about 80 to 100 bases in length. In addition to the detection of mutations, these probes can be used to discover the Zcytor11 gene in other mammalian species. The probes can either be positive strand or anti-sense strands, and they can be comprised of DNA or RNA.

20 These and other aspects of the invention will become evident upon reference to the following detailed description and the attached drawing.

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DETAILED DESCRIPTION OF THE INVENTION

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation
30 arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also
35 used herein to denote a protein encoded by an allelic variant of a gene.

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The term "expression vector" is used to denote a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription.

5 Such additional segments include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from plasmid or viral DNA, or may
10 contain elements of both.

The term "isolated", when applied to a polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free
15 of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems.

"Operably linked", when referring to DNA
20 segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

25 A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a
30 combination of natural and synthetic molecules.

The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of
35 RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

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The term "receptor" is used herein to denote a cell-associated protein, or a polypeptide subunit of such a protein, that binds to a bioactive molecule (the "ligand") and mediates the effect of the ligand on the cell. Binding of ligand to receptor results in a conformational change in the receptor (and, in some cases, receptor multimerization, i.e., association of identical or different receptor subunits) that causes interactions between the effector domain(s) and other molecule(s) in the cell. These interactions in turn lead to alterations in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, cell proliferation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. The term "receptor polypeptide" is used to denote complete receptor polypeptide chains and portions thereof, including isolated functional domains (e.g., ligand-binding domains).

A "secretory signal sequence" is a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

A "soluble receptor" is a receptor polypeptide that is not bound to a cell membrane. Soluble receptors are most commonly ligand-binding receptor polypeptides that lack transmembrane and cytoplasmic domains. Soluble receptors can comprise additional amino acid residues, such as affinity tags that provide for purification of the

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polypeptide or provide sites for attachment of the polypeptide to a substrate, or immunoglobulin constant region sequences. Many cell-surface receptors have naturally occurring, soluble counterparts that are produced by proteolysis or translated from alternatively spliced mRNAs. Receptor polypeptides are said to be substantially free of transmembrane and intracellular polypeptide segments when they lack sufficient portions of these segments to provide membrane anchoring or signal transduction, respectively.

Analysis of the tissue distribution of the mRNA corresponding to this novel DNA showed that mRNA level was highest in pancreas, followed by a much lower levels in thymus, colon and small intestine. The receptor has been designated "Zcytor11".

Cytokine receptors subunits are characterized by a multi-domain structure comprising a ligand-binding domain and an effector domain that is typically involved in signal transduction. Multimeric cytokine receptors include homodimers (e.g., PDGF receptor $\alpha\alpha$ and $\beta\beta$ isoforms, erythropoietin receptor, MPL [thrombopoietin receptor], and G-CSF receptor), heterodimers whose subunits each have ligand-binding and effector domains (e.g., PDGF receptor $\alpha\beta$ isoform), and multimers having component subunits with disparate functions (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and GM-CSF receptors). Some receptor subunits are common to a plurality of receptors. For example, the AIC2B subunit, which cannot bind ligand on its own but includes an intracellular signal transduction domain, is a component of IL-3 and GM-CSF receptors. Many cytokine receptors can be placed into one of four related families on the basis of their structures and functions. Class I hematopoietic receptors, for example, are characterized by the presence of a domain containing conserved cysteine residues and the WSXWS

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motif. Additional domains, including protein kinase domains; fibronectin type III domains; and immunoglobulin domains, which are characterized by disulfide-bonded loops, are present in certain hematopoietic receptors.

5 Cytokine receptor structure has been reviewed by Urdal, Ann. Reports Med. Chem. 26:221-228, 1991 and Cosman, Cytokine 5:95-106, 1993. It is generally believed that under selective pressure for organisms to acquire new biological functions, new receptor family members arose from duplication of existing receptor genes leading to the
10 existence of multi-gene families. Family members thus contain vestiges of the ancestral gene, and these characteristic features can be exploited in the isolation and identification of additional family members.

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Cell-surface cytokine receptors are further characterized by the presence of additional domains. These receptors are anchored in the cell membrane by a transmembrane domain characterized by a sequence of
20 hydrophobic amino acid residues (typically about 21-25 residues), which is commonly flanked by positively charged residues (Lys or Arg). On the opposite end of the protein from the extracellular domain and separated from it by the transmembrane domain is an intracellular domain.

25

The novel receptor of the present invention, Zcytor11, is a class II cytokine receptor. These receptors usually bind to four-helix-bundle cytokines. Interleukin-10 and the interferons have receptors in this class (e.g.,
30 interferon-gamma alpha and beta chains and the interferon-alpha/beta receptor alpha and beta chains). Class II cytokine receptors are characterized by the presence of one or more cytokine receptor modules (CRM) in their extracellular domains. The CRMs of class II cytokine
35 receptors are somewhat different than the better known CRMs of class I cytokine receptors. While the class II

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CRMs contain two type-III fibronectin-like domains, they differ in organization.

Zcytor11, like all known class II receptors
5 except interferon-alpha/beta receptor alpha chain, has only a single class II CRM in its extracellular domain. Zcytor11 appears to be a receptor for a helical cytokine of the interferon/IL-10 class. Using the Zcytor11 receptor we can identify ligands and additional compounds which
10 would be of significant therapeutic value.

As was stated above, Zcytor11 is similar to the interferon α receptor α chain. Uze *et al.* *Cell* 60 255-264 (1996) Analysis of a human cDNA clone encoding Zcytor11
15 (SEQ ID NO:1) revealed an open reading frame encoding 574 amino acids (SEQ ID NO:2) comprising an extracellular ligand-binding domain of approximately 211 amino acid residues (residues 18-228 of SEQ ID NO:2), a transmembrane domain of approximately 23 amino acid residues (residues
20 229-251 of SEQ ID NO:2), and an intracellular domain of approximately 313 amino acid residues (residues 252 to 574 of SEQ ID NO:2). Those skilled in the art will recognize that these domain boundaries are approximate and are based on alignments with known proteins and predictions of
25 protein folding. Deletion of residues from the ends of the domains is possible.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar
30 sized regions of SEQ ID NO:1 or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is
35 the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typical stringent conditions are those in

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which the salt concentration is at least about 0.02 M at pH 7 and the temperature is at least about 60°C. As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for
5 isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from pancreas or prostate tissues although cDNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction
10 followed by isolation by centrifugation in a CsCl gradient [Chirgwin et al., *Biochemistry* 18:52-94, (1979)]. Poly(A)⁺ RNA is prepared from total RNA using the method of Aviv and Leder *Proc. Natl. Acad. Sci. USA* 69:1408-1412, (1972). Complementary DNA (cDNA) is prepared from
15 poly(A)⁺ RNA using known methods. Polynucleotides encoding Zcytor11 polypeptides are then identified and isolated by, for example, hybridization or PCR.

Those skilled in the art will recognize that the
20 sequences disclosed in SEQ ID NOS:1 and 2 represent single alleles of the human Zcytor11 receptor. Allelic variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures.

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The present invention further provides counterpart receptors and polynucleotides from other species ("species orthologs"). Of particular interest are Zcytor11 receptors from other mammalian species, including
30 murine, porcine, ovine, bovine, canine, feline, equine, and non-human primates. Species orthologs of the human Zcytor11 receptor can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For
35 example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the receptor. Suitable sources of mRNA can be identified by probing Northern

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blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A receptor-encoding cDNA can then be isolated by a variety of methods, such as by
5 probing with a complete or partial cDNA of human and other primates or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent No. 4,683,202), using primers
10 designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to the receptor. Similar techniques can also be applied to the
15 isolation of genomic clones.

The present invention also provides isolated receptor polypeptides that are substantially homologous to the receptor polypeptide of SEQ ID NO: 2. By "isolated"
20 is meant a protein or polypeptide that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal
25 origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. The term "substantially homologous" is used herein to denote polypeptides having 50%, preferably 60%, more preferably at least 80%,
30 sequence identity to the sequences shown in SEQ ID NO:2,. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2. Percent sequence identity is determined by conventional methods. See, for example, *Altschul et al.*,
35 *Bull. Math. Bio.* 48: 603-616, (1986) and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919, (1992). Briefly, two amino acid sequences are aligned to

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optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the ^{BLOSUM}~~"Blossum 62"~~ scoring matrix of Henikoff and Henikoff (id.) as shown in Table 2 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

Total number of identical matches

x 100

[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]

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[illegible]

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Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

5 Substantially homologous proteins and polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 3) and other substitutions
10 that do not significantly affect the folding or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about
15 20-25 residues, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, protein A [Nilsson et al., *EMBO J.* 4:1075, (1985); Nilsson et al., *Methods Enzymol.* 198:3, (1991)], glutathione S transferase [Smith and Johnson, *Gene* 67:31, 20 1988), or other antigenic epitope or binding domain. See, in general Ford et al., Protein Expression and Purification 2: 95-107, (1991). DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

²⁵
7160X Table 3

Conservative amino acid substitutions

30	Basic:	arginine
		lysine
		histidine
	Acidic:	glutamic acid
		aspartic acid
	Polar:	glutamine
		asparagine

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Table 3, continued

	Hydrophobic:	leucine
		isoleucine
		valine
5	Aromatic:	phenylalanine
		tryptophan
		tyrosine
	Small:	glycine
10		alanine
		serine
		threonine
		methionine
15	Essential amino acids in the receptor	
	polypeptides of the present invention can be identified	
	according to procedures known in the art, such as site-	
	directed mutagenesis or alanine-scanning mutagenesis	
	[Cunningham and Wells, <i>Science</i> 244, 1081-1085, (1989);	
20	Bass et al., <i>Proc. Natl. Acad. Sci. USA</i> 88:4498-4502,	
	(1991)]. In the latter technique, single alanine	
	mutations are introduced at every residue in the molecule,	
	and the resultant mutant molecules are tested for	
	biological activity (e.g., ligand binding and signal	
25	transduction) to identify amino acid residues that are	
	critical to the activity of the molecule. Sites of	
	ligand-receptor interaction can also be determined by	
	analysis of crystal structure as determined by such	
	techniques as nuclear magnetic resonance, crystallography	
30	or photoaffinity labeling. See, for example, de Vos et	
	al., <i>Science</i> 255:306-312, (1992); Smith et al., <i>J. Mol.</i>	
	<i>Biol.</i> 224:899-904, (1992); Wlodaver et al., <i>FEBS Lett.</i>	
	309:59-64, (1992)]. The identities of essential amino	
	acids can also be inferred from analysis of homologies	
35	with related receptors.	

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Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer *Science* 241:53-57, (1988) or Bowie and Sauer *Proc. Natl. Acad. Sci. USA* 86:2152-2156, (1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display e.g., Lowman et al., *Biochem.* 30:10832-10837, (1991); Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis [Derbyshire et al., *Gene* 46:145, (1986); Ner et al., *DNA* 7:127, (1988)].

Mutagenesis methods as disclosed above can be combined with high-throughput screening methods to detect activity of cloned, mutagenized receptors in host cells. Preferred assays in this regard include cell proliferation assays and biosensor-based ligand-binding assays, which are described below. Mutagenized DNA molecules that encode active receptors or portions thereof (e.g., ligand-binding fragments) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

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Using the methods discussed above, one of ordinary skill in the art can prepare a variety of polypeptides that are substantially homologous to residues 18 to 228 of SEQ ID NO:2 or allelic variants thereof and retain the ligand-binding properties of the wild-type receptor. Such polypeptides may include additional amino acids from an extracellular ligand-binding domain of a

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Zcytor11 receptor as well as part or all of the transmembrane and intracellular domains. Such polypeptides may also include additional polypeptide segments as generally disclosed above.

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The receptor polypeptides of the present invention, including full-length receptors, receptor fragments (e.g. ligand-binding fragments), and fusion polypeptides can be produced in genetically engineered
10 host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured
15 cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory
20 Press, Cold Spring Harbor, NY, (1989), and Ausubel *et al.*, *ibid.*, which are incorporated herein by reference.

In general, a DNA sequence encoding a Zcytor11 receptor polypeptide is operably linked to other genetic
25 elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will
30 recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter
35 of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

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To direct a Zcytor11 receptor polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the receptor, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is joined to the Zcytor11 DNA sequence in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Cultured mammalian cells are preferred hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection [Wigler et al., *Cell* 14:725, (1978); Corsaro and Pearson, *Somatic Cell Genetics* 7:603, (1981); Graham and Van der Eb, *Virology* 52:456, (1973)], electroporation [Neumann et al., *EMBO J.* 1:841-845, (1982)], DEAE-dextran mediated transfection [Ausubel et al., eds., *Current Protocols in Molecular Biology*, (John Wiley and Sons, Inc., NY, 1987), and liposome-mediated transfection (Hawley-Nelson et al., *Focus* 15:73, (1993); Ciccarone et al., *Focus* 15:80, (1993)], which are incorporated herein by reference. The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., *J. Gen.*

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Virol. 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, 5 Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 10 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as 15 "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. 20 Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by 25 culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate 30 reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

35 Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. Transformation of insect cells and production of

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foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222; Bang et al., U.S. Patent No. 4,775,624; and WIPO publication WO 94/06463, which are incorporated herein by reference. The use of

- 5 *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., *J. Biosci. (Bangalore)* 11:47-58, (1987).

- Fungal cells, including yeast cells, and
- 10 particularly cells of the genus *Saccharomyces*, can also be used within the present invention, such as for producing receptor fragments or polypeptide fusions. Methods for transforming yeast cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for
- 15 example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the
- 20 selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the POT1 vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to
- 25 be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No.
- 30 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago*
- 35 *maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., *J. Gen. Microbiol.*

132:3459-3465, (1986) and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

Within one aspect of the present invention, a novel receptor is produced by a cultured cell, and the cell is used to screen for ligands for the receptor, including the natural ligand, as well as agonists and antagonists of the natural ligand. To summarize this approach, a cDNA or gene encoding the receptor is combined with other genetic elements required for its expression (e.g., a transcription promoter), and the resulting expression vector is inserted into a host cell. Cells that express the DNA and produce functional receptor are selected and used within a variety of screening systems.

Mammalian cells suitable for use in expressing Zcytor11 receptors and transducing a receptor-mediated

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signal include cells that express other receptor subunits which may form a functional complex with Zcytor11. These subunits may include those of the interferon receptor family or of other class II or class I cytokine receptors.

5 It is also preferred to use a cell from the same species as the receptor to be expressed. Within a preferred embodiment, the cell is dependent upon an exogenously supplied hematopoietic growth factor for its proliferation. Preferred cell lines of this type are the
10 human TF-1 cell line (ATCC number CRL-2003) and the AML-193 cell line (ATCC number CRL-9589), which are GM-CSF-dependent human leukemic cell lines and BaF3 [Palacios and Steinmetz, *Cell* 41: 727-734, (1985)] which is an IL-3 dependent murine pre-B cell line. Other cell lines include
15 BHK, COS-1 and CHO cells.

Suitable host cells can be engineered to produce the necessary receptor subunits or other cellular component needed for the desired cellular response. This
20 approach is advantageous because cell lines can be engineered to express receptor subunits from any species, thereby overcoming potential limitations arising from species specificity. Species orthologs of the human receptor cDNA can be cloned and used within cell lines
25 from the same species, such as a mouse cDNA in the BaF3 cell line. Cell lines that are dependent upon one hematopoietic growth factor, such as GM-CSF or IL-3, can thus be engineered to become dependent upon a Zcytor11 ligand.

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Cells expressing functional receptor are used within screening assays. A variety of suitable assays are known in the art. These assays are based on the detection of a biological response in a target cell. One such assay
35 is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound, and cell proliferation is detected by, for example, measuring

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incorporation of tritiated thymidine or by colorimetric assay based on the metabolic breakdown of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) [Mosman, *J. Immunol. Meth.* 65: 55-63, (1983)]. An alternative assay format uses cells that are further engineered to express a reporter gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. A preferred promoter element in this regard is a serum response element, or SRE. See, e.g., Shaw et al., *Cell* 56:563-572, (1989). A preferred such reporter gene is a luciferase gene [de Wet et al., *Mol. Cell. Biol.* 7:725, (1987)]. Expression of the luciferase gene is detected by luminescence using methods known in the art [e.g., Baumgartner et al., *J. Biol. Chem.* 269:29094-29101, (1994); Schenborn and Goiffin, *Promega_Notes* 41:11, 1993]. Luciferase activity assay kits are commercially available from, for example, Promega Corp., Madison, WI. Target cell lines of this type can be used to screen libraries of chemicals, cell-conditioned culture media, fungal broths, soil samples, water samples, and the like. For example, a bank of cell-conditioned media samples can be assayed on a target cell to identify cells that produce ligand. Positive cells are then used to produce a cDNA library in a mammalian expression vector, which is divided into pools, transfected into host cells, and expressed. Media samples from the transfected cells are then assayed, with subsequent division of pools, re-transfection, subculturing, and re-assay of positive cells to isolate a cloned cDNA encoding the ligand.

A natural ligand for the Zcytor11 receptor can also be identified by mutagenizing a cell line expressing the receptor and culturing it under conditions that select for autocrine growth. See WIPO publication WO 95/21930. Within a typical procedure, IL-3 dependent BaF3 cells

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expressing Zcytor11 and the necessary additional subunits are mutagenized, such as with 2-ethylmethanesulfonate (EMS). The cells are then allowed to recover in the presence of IL-3, then transferred to a culture medium lacking IL-3 and IL-4. Surviving cells are screened for the production of a Zcytor11 ligand, such as by adding soluble receptor to the culture medium or by assaying conditioned media on wild-type BaF3 cells and BaF3 cells expressing the receptor.

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An additional screening approach provided by the present invention includes the use of hybrid receptor polypeptides. These hybrid polypeptides fall into two general classes. Within the first class, the intracellular domain of Zcytor11, comprising approximately residues 252 to 574 of SEQ ID NO:2, is joined to the ligand-binding domain of a second receptor. It is preferred that the second receptor be a hematopoietic cytokine receptor, such as mpl receptor [Souyri *et al.*, Cell, 63: 1137-1147, (1990)]. The hybrid receptor will further comprise a transmembrane domain, which may be derived from either receptor. A DNA construct encoding the hybrid receptor is then inserted into a host cell. Cells expressing the hybrid receptor are cultured in the presence of a ligand for the binding domain and assayed for a response. This system provides a means for analyzing signal transduction mediated by Zcytor11 while using readily available ligands. This system can also be used to determine if particular cell lines are capable of responding to signals transduced by Zcytor11. A second class of hybrid receptor polypeptides comprise the extracellular (ligand-binding) domain of Zcytor11 (approximately residues 18 to 228 of SEQ ID NO:2) with an intracellular domain of a second receptor, preferably a hematopoietic cytokine receptor, and a transmembrane domain. Hybrid receptors of this second class are expressed in cells known to be capable of responding to

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signals transduced by the second receptor. Together, these two classes of hybrid receptors enable the identification of a responsive cell type for the development of an assay for detecting a Zcytor11 ligand.

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Cells found to express the ligand are then used to prepare a cDNA library from which the ligand-encoding cDNA can be isolated as disclosed above. The present invention thus provides, in addition to novel receptor polypeptides, methods for cloning polypeptide ligands for the receptors.

The tissue specificity of Zcytor11 expression suggests a role in the development of the pancreas, small intestine, colon and the thymus. In view of the tissue specificity observed for this receptor, agonists (including the natural ligand) and antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as receptor agonists are useful for stimulating proliferation and development of target cells *in vitro* and *in vivo*. For example, agonist compounds are useful as components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists or antagonist may be useful in specifically regulating the growth and/or development of pancreatic, gastro-intestinal or thymic-derived cells in culture. These compounds are useful as research reagents for characterizing sites of ligand-receptor interaction. *In vivo*, receptor agonists or antagonists may find application in the treatment pancreatic, gastro-intestinal or thymic diseases.

Agonists or antagonists to Zcytor11 may include small families of peptides. These peptides may be identified employing affinity selection conditions that are known in the art, from a population of candidates

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present in a peptide library. Peptide libraries include combinatorial libraries chemically synthesized and presented on solid support [Lam et al., *Nature* 354: 82-84 (1991)] or are in solution [Houghten et al., *BioTechniques* 13: 412-421, (1992)], expressed then linked to plasmid DNA [Cull et al., *Proc. Natl. Acad. Sci. USA* 89: 1865-1869 (1992)] or expressed and subsequently displayed on the surfaces of viruses or cells [Boder and Wittrup, *Nature Biotechnology* 15: 553-557(1997); Cwirla et al. *Science* 276: 1696-1699 (1997)].

Zcytor11 may also be used within diagnostic systems for the detection of circulating levels of ligand. Within a related embodiment, antibodies or other agents that specifically bind to Zcytor11 can be used to detect circulating receptor polypeptides. Elevated or depressed levels of ligand or receptor polypeptides may be indicative of pathological conditions, including cancer.


Zcytor11 receptor polypeptides can be prepared by expressing a truncated DNA encoding the extracellular domain, for example, a polypeptide which contains residues 18 through 228 of a human Zcytor11 receptor (SEQ ID NO:2 or the corresponding region of a non-human receptor. It is preferred that the extracellular domain polypeptides be prepared in a form substantially free of transmembrane and intracellular polypeptide segments. For example, the C-terminus of the receptor polypeptide may be at residue 228 of SEQ ID NO:2 or the corresponding region of an allelic variant or a non-human receptor. To direct the export of the receptor domain from the host cell, the receptor DNA is linked to a second DNA segment encoding a secretory peptide, such as a t-PA secretory peptide. To facilitate purification of the secreted receptor domain, a C-terminal extension, such as a poly-histidine tag, substance P, Flag™ peptide [Hopp et al., *Biotechnology* 6:1204-1210, (1988); available from Eastman Kodak Co., New Haven, CT] or

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another polypeptide or protein for which an antibody or other specific binding agent is available, can be fused to the receptor polypeptide.

5 In an alternative approach, a receptor
extracellular domain can be expressed as a fusion with
immunoglobulin heavy chain constant regions, typically an
F_C fragment, which contains two constant region domains
and a hinge region but lacks the variable region. Such
10 fusions are typically secreted as multimeric molecules
wherein the Fc portions are disulfide bonded to each other
and two receptor polypeptides are arrayed in closed
proximity to each other. Fusions of this type can be used
to affinity purify the cognate ligand from solution, as an
15 *in vitro* assay tool, to block signals *in vitro* by
specifically titrating out ligand, and as antagonists *in*
vivo by administering them parenterally to bind
circulating ligand and clear it from the circulation. To
purify ligand, a Zcytor11-Ig chimera is added to a sample
20 containing the ligand (e.g., cell-conditioned culture
media or tissue extracts) under conditions that facilitate
receptor-ligand binding (typically near-physiological
temperature, pH, and ionic strength). The chimera-ligand
complex is then separated by the mixture using protein A,
25 which is immobilized on a solid support (e.g., insoluble
resin beads). The ligand is then eluted using
conventional chemical techniques, such as with a salt or
pH gradient. In the alternative, the chimera itself can
be bound to a solid support, with binding and elution
30 carried out as above. The chimeras may be used *in vivo* to
regulate gastrointestinal, pancreatic or thymic functions.
Chimeras with high binding affinity are administered
parenterally (e.g., by intramuscular, subcutaneous or
intravenous injection). Circulating molecules bind ligand
35 and are cleared from circulation by normal physiological
processes. For use in assays, the chimeras are bound to a
support via the F_C region and used in an ELISA format.



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A preferred assay system employing a ligand-binding receptor fragment uses a commercially available biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, NJ), wherein the receptor fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, *J. Immunol. Methods* 145:229-240, (1991) and Cunningham and Wells, *J. Mol. Biol.* 234:554-563, (1993). A receptor fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If ligand is present in the sample, it will bind to the immobilized receptor polypeptide, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

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Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity. See, Scatchard, *Ann. NY Acad. Sci.* 51: 660-672, (1949) and calorimetric assays [Cunningham et al., *Science* 253:545-548, (1991); Cunningham et al., *Science* 254:821-825, (1991)].

A receptor ligand-binding polypeptide can also be used for purification of ligand. The receptor polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-

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hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting media will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration or pH to disrupt ligand-receptor binding.

10 Zcytor11 polypeptides can also be used to prepare antibodies that specifically bind to Zcytor11 polypeptides. As used herein, the term "antibodies" includes polyclonal antibodies, monoclonal antibodies, single-chain antibodies and antigen-binding fragments thereof such as F(ab')₂ and Fab fragments, and the like, including genetically engineered antibodies. Antibodies are defined to be specifically binding if they bind to a Zcytor11 polypeptide with a K_a of greater than or equal to $10^7/M$. The affinity of a monoclonal antibody can be readily determined by one of ordinary skill in the art (see, for example, Scatchard, *ibid.*).

 Methods for preparing polyclonal and monoclonal antibodies are well known in the art. See for example, 25 Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, NY, (1989); and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press, Inc., Boca Raton, FL, (1982), which are incorporated herein by reference. 30 As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats. The immunogenicity of a Zcytor11 polypeptide may be increased 35 through the use of an adjuvant such as Freund's complete or incomplete adjuvant. A variety of assays known to those skilled in the art can be utilized to detect

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antibodies which specifically bind to Zcytor11 polypeptides. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, (1988).

- 5 Representative examples of such assays include: concurrent immunoelectrophoresis, radio-immunoassays, radio-immunoprecipitations, enzyme-linked immunosorbent assays (ELISA), dot blot assays, inhibition or competition assays, and sandwich assays.

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Antibodies to Zcytor11 may be used for tagging cells that express the receptor, for affinity purification, within diagnostic assays for determining circulating levels of soluble receptor polypeptides, and
15 as antagonists to block ligand binding and signal transduction *in vitro* and *in vivo*.

- Anti-idiotypic antibodies which bind to the antigenic binding site of antibodies to Zcytor11 are also
20 considered part of the present invention. The antigenic binding region of the anti-idiotypic antibody thus will mimic the ligand binding region of Zcytor11. An anti-idiotypic antibody thus could be used to screen for possible ligands of the Zcytor11 receptor. Thus
25 neutralizing antibodies to Zcytor11 can be used to produce anti-idiotypic antibodies by methods well known in the art as is described in, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual, Second Edition*, (Cold Spring Harbor, NY, 1989); and Hurrell, J. G. R.,
30 Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, (CRC Press, Inc., Boca Raton, FL, 1982).

- Zcytor11 maps 84.62 cR from the top of the human chromosome a linkage group on the WICGR radiation hybrid
35 map. The use of surrounding markers positioned Zcytor11 in the 1p35.2 to 35.1 region.

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Thus Zcytor11 could be used to generate a probe that could allow detection of an aberration of the Zcytor11 gene in the 1p chromosome which may indicate the presence of a cancerous cells or a predisposition to cancerous cell development. This region of chromosome 1 is frequently involved in visible deletions or loss of heterozygosity in tumors derived from the neural crest cells particularly neuroblastomas and melanomas. For further discussions on developing polynucleotide probes and hybridization see *Current Protocols in Molecular Biology* Ausubel, F. et al. Eds. (John Wiley & Sons Inc. 1991).

The invention is further illustrated by the following non-limiting examples.

Example 1

Production a Pancreatic Islet Cell cDNA Library

Zcytor11 was cloned from a pancreatic islet cell cDNA library produced according to the following procedure. RNA extracted from pancreatic islet cells was reversed transcribed in the following manner. The first strand cDNA reaction contained 10 µl of human pancreatic islet cell poly d(T)-selected poly (A)⁺ mRNA (Clontech, Palo Alto, CA) at a concentration of 1.0 mg/ml, and 2 µl of 20 pmole/µl first strand primer ZC6171 (SEQ ID NO: 6) containing an Xho I restriction site. The mixture was heated at 70°C for 2.5 minutes and cooled by chilling on ice. First strand cDNA synthesis was initiated by the addition of 8 µl of first strand buffer (5x SUPERScript® buffer; Life Technologies, Gaithersburg, MD), 4 µl of 100 mM dithiothreitol, and 3 µl of a deoxynucleotide triphosphate (dNTP) solution containing 10 mM each of dTTP, dATP, dGTP and 5-methyl-dCTP (Pharmacia LKB Biotechnology, Piscataway, NJ) to the RNA-primer mixture. The reaction mixture was incubated at 40° C for 2 minutes, followed by the addition of 10 µl of 200 U/µl RNase H⁻

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reverse transcriptase (SUPERScript II®; Life Technologies). The efficiency of the first strand synthesis was analyzed in a parallel reaction by the addition of 10 µCi of ³²P-αdCTP to a 5 µl aliquot from one of the reaction mixtures to label the reaction for analysis. The reactions were incubated at 40°C for 5 minutes, 45°C for 50 minutes, then incubated at 50°C for 10 minutes. Unincorporated ³²P-αdCTP in the labeled reaction was removed by chromatography on a 400 pore size gel filtration column (Clontech Laboratories, Palo Alto, CA). The unincorporated nucleotides and primers in the unlabeled first strand reactions were removed by chromatography on 400 pore size gel filtration column (Clontech Laboratories, Palo Alto, CA). The length of labeled first strand cDNA was determined by agarose gel electrophoresis.

The second strand reaction contained 102 µl of the unlabeled first strand cDNA, 30 µl of 5x polymerase I buffer (125 mM Tris: HCl, pH 7.5, 500 mM KCl, 25 mM MgCl₂, 50mM (NH₄)₂SO₄), 2.0 µl of 100 mM dithiothreitol, 3.0 µl of a solution containing 10 mM of each deoxynucleotide triphosphate, 7 µl of 5 mM β-NAD, 2.0 µl of 10 U/µl *E. coli* DNA ligase (New England Biolabs; Beverly, MA), 5 µl of 10 U/µl *E. coli* DNA polymerase I (New England Biolabs, Beverly, MA), and 1.5 µl of 2 U/µl RNase H (Life Technologies, Gaithersburg, MD). A 10 µl aliquot from one of the second strand synthesis reactions was labeled by the addition of 10 µCi ³²P-αdCTP to monitor the efficiency of second strand synthesis. The reactions were incubated at 16° C for two hours, followed by the addition of 1 µl of a 10 mM dNTP solution and 6.0 µl T4 DNA polymerase (10 U/µl, Boehringer Mannheim, Indianapolis, IN) and incubated for an additional 10 minutes at 16°C. Unincorporated ³²P-αdCTP in the labeled reaction was removed by chromatography through a 400 pore size gel filtration column (Clontech Laboratories, Palo Alto, CA)

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before analysis by agarose gel electrophoresis. The reaction was terminated by the addition of 10.0 µl 0.5 M EDTA and extraction with phenol/chloroform and chloroform followed by ethanol precipitation in the presence of 3.0 M Na acetate and 2 µl of Pellet Paint carrier (Novagen, Madison, WI). The yield of cDNA was estimated to be approximately 2 µg from starting mRNA template of 10 µg.

Eco RI adapters were ligated onto the 5' ends of the cDNA described above to enable cloning into an expression vector. A 12.5 µl aliquot of cDNA (~2.0 µg) and 3 µl of 69 pmole/µl of *Eco* RI adapter (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) were mixed with 2.5 µl 10x ligase buffer (660 mM Tris-HCl pH 7.5, 100 mM MgCl₂), 2.5 µl of 10 mM ATP, 3.5 µl 0.1 M DTT and 1 µl of 15 U/µl T4 DNA ligase (Promega Corp., Madison, WI). The reaction was incubated 1 hour at 5°C, 2 hours at 7.5°C, 2 hours at 10°C, 2 hours at 12.5°C and 16 hours at 10° C. The reaction was terminated by the addition of 65 µl H₂O and 10 µl 10X H buffer (Boehringer Mannheim, Indianapolis, IN) and incubation at 70°C for 20 minutes.

To facilitate the directional cloning of the cDNA into an expression vector, the cDNA was digested with *Xho* I, resulting in a cDNA having a 5' *Eco* RI cohesive end and a 3' *Xho* I cohesive end. The *Xho* I restriction site at the 3' end of the cDNA had been previously introduced. Restriction enzyme digestion was carried out in a reaction mixture by the addition of 1.0 µl of 40 U/µl *Xho* I (Boehringer Mannheim, Indianapolis, IN). Digestion was carried out at 37°C for 45 minutes. The reaction was terminated by incubation at 70°C for 20 minutes and chromatography through a 400 pore size gel filtration column (Clontech Laboratories, Palo Alto, CA).

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The cDNA was ethanol precipitated, washed with 70% ethanol, air dried and resuspended in 10.0 µl water, 2

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μl of 10X kinase buffer (660 mM Tris-HCl, pH 7.5, 100 mM MgCl₂), 0.5 μl 0.1 M DTT, 2 μl 10 mM ATP, 2 μl T4 polynucleotide kinase (10 U/μl, Life Technologies, Gaithersburg, MD). Following incubation at 37° C for 30 minutes, the cDNA was ethanol precipitated in the presence of 2.5 M Ammonium Acetate, and electrophoresed on a 0.8% low melt agarose gel. The contaminating adapters and cDNA below 0.6 Kb in length were excised from the gel. The electrodes were reversed, and the cDNA was electrophoresed until concentrated near the lane origin. The area of the gel containing the concentrated cDNA was excised and placed in a microfuge tube, and the approximate volume of the gel slice was determined. An aliquot of water approximately three times the volume of the gel slice (300 μl) and 35 μl 10x β-agarose I buffer (New England Biolabs) was added to the tube, and the agarose was melted by heating to 65°C for 15 minutes. Following equilibration of the sample to 45°C, 3 μl of 1 U/μl β-agarose I (New England Biolabs, Beverly, MA) was added, and the mixture was incubated for 60 minutes at 45°C to digest the agarose. After incubation, 40 μl of 3 M Na acetate was added to the sample, and the mixture was incubated on ice for 15 minutes. The sample was centrifuged at 14,000 x g for 15 minutes at room temperature to remove undigested agarose. The cDNA was ethanol precipitated, washed in 70% ethanol, air-dried and resuspended in 40 μl water.

Following recovery from low-melt agarose gel, the cDNA was cloned into the *Eco* RI and *Xho* I sites of pBLUESCRIPT SK+ vector (Gibco/BRL, Gaithersburg, MD) and electroporated into DH10B cells. Bacterial colonies containing ESTs of known genes were identified and eliminated from sequence analysis by reiterative cycles of probe hybridization to hi-density colony filter arrays (Genome Systems, St. Louis, MI). cDNAs of known genes were pooled in groups of 50 - 100 inserts and were labeled with ³²P-αdCTP using a MEGAPRIME labeling kit (Amersham,

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Arlington Heights, IL). Colonies which did not hybridize to the probe mixture were selected for sequencing. Sequencing was done using an ABI 377 sequencer using either the T3 or the reverse primer. The resulting data were analyzed which resulted in the identification of EST LISF104376 (SEQ ID NO: 3).

Example 2.

10 Cloning of Zcytor11

Expressed sequence tag (EST) LISF104376 (SEQ ID NO:3) contained in plasmid pSLIS4376 was isolated from a human pancreatic islet cell cDNA library. Following sequencing of the entire pSLIS4376 cDNA insert, it was determined not to encode a full-length Zcytor11 polypeptide.

A full length Zcytor11 encoding cDNA was isolated by screening a human islet cDNA library using a probe that was generated by PCR primers ZC14,295 (SEQ ID NO:4) and ZC14294 (SEQ ID NO:5) and the pSLIS4376 template. (For details on the construction of the pancreatic islet cell cDNA library, see Example 2 below.) The resulting probe of 276 bp containing nucleotides 142 to 417 of SEQ ID NO:1 was purified by chromatography through a 100 pore size spin column (Clontech, Palo Alto, CA). The purified probe was labeled with ³²P-αCTP using a MEGAPRIME® labeling kit (Amersham Corp., Arlington Heights, IL). The labeled probe was purified on a NUCTRAP® purification column (Stratagene Cloning Systems, La Jolla, CA) for library screening.

Following recovery of the islet cDNA from a low-melt agarose gel from Example 1, the cDNA was cloned into the Eco RI and Xho I sites; of pBLUESCRIPT SK+ (Gibco/BRL, Gaithersburg, MD) and electroporated into DH10B cells. Bacterial clones from resulting cDNA library were

individually placed on a grid of a high-density colony filter arrays (Genome Systems, St. Louis, MI) and were probed with the labeled Zcytor11 probe described above. A glycerol stock of each clone on each grid was also made to expedite the isolation of positive clones. The filters were first pre-washed in an aqueous solution containing 0.25X standard sodium citrate (SSC), 0.25% sodium dodecyl sulfate (SDS) and 1 mM EDTA to remove cellular debris and then prehybridized in a hybridization solution (5X SSC, 5X Denhardt's solution, 0.2% SDS and 1 mM EDTA) containing 100 µg/ml heat-denature, sheared salmon sperm DNA).

Fifty nanograms of the PCR-derived Zcytor11 probe was radiolabeled with ^{32}P - α CTP by random priming using the MEGAPRIME® DNA labeling system (Amersham, Arlington Heights, IL). The prehybridization solution was replaced with fresh hybridization containing 1×10^6 cpm/ml probe and allowed to hybridize at 65° C overnight. The filters were washed in a wash buffer containing 0.25X SSC, 0.25% SDS and 1 mM EDTA at 65° C.

Following autoradiography, three signals were detected among 40,000 clones on the grids of the filter array. From the grid coordinates of the positive signals, the corresponding clones, pSLR11-1, pSLR11-2 and pSLR11-3 were retrieved from the glycerol stock and their inserts sequenced. The insert in pSLR11-1 was determined to be 2831 base pairs (bp) and encoded full-length Zcytor11 polypeptide.

Example 3

Expression of Human Zcytor11 mRNA in Human Tissues

Poly(A)⁺ RNAs isolated brain, colon, heart, kidney, liver, lung, ovary, pancreas, prostate, placenta, peripheral blood leukocytes, stomach, spleen, skeletal

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muscle, small intestine, testis, thymus, thyroid, spinal
cord, lymph node, trachea, adrenal gland and bone marrow
were hybridized under high stringency conditions with a
radiolabeled DNA probe containing nucleotides 181-456 of
5 (SEQ ID NO:1). Membranes were purchased from Clontech. The
membrane were washed with 0.1X SSC, 0.1% SDS at 50°C and
autoradiographed for 24 hours. The mRNA levels were
highest in pancreas with low levels in colon, small
intestine and thymus. The receptor mRNA localization
10 suggests that Zcytor11 may regulate gastrointestinal,
pancreatic or thymic functions.

Example 4

15 Chromosomal Assignment and Placement of Zcytor11

Zcytor11 was mapped to chromosome 1 using the
commercially available version of the Whitehead
Institute/MIT Center for Genome Research's "GeneBridge 4
20 Radiation Hybrid Panel" (Research Genetics, Inc.,
Huntsville, AL). The GeneBridge 4 Radiation Hybrid Panel
contains PCRable DNAs from each of 93 radiation hybrid
clones, plus two control DNAs (the HFL donor and the A23
recipient). A publicly available WWW server ([http://www-](http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl)
25 [genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl](http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl)) allows
mapping relative to the Whitehead Institute/MIT Center for
Genome Research's radiation hybrid map of the human genome
(the "WICGR" radiation hybrid map) which was constructed
with the GeneBridge 4 Radiation Hybrid Panel.

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